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Research Article
Potential of Organic Mercury-resistant Bacteria Isolated from Mercury Contaminated Sites for Organic Mercury Remediation

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Abstract

Background and Objective: Efforts at organic mercury detoxification can be carried out using resistant bacteria that can live in an environment contaminated with the compound. This study aimed at isolating and identifying resistant bacteria from mercury-contaminated environments and analysing their ability to detoxify organic mercury. **Materials and Methods:** Soil samples were obtained from 3 gold processing locations that make use of mercury in Tanoyan Village, Bolaang Mongondow district, North Sulawesi province. The identification was carried out on the mercury-resistant bacteria through morphological and molecular tests. Bacteria which were highly resistant to mercury were examined for their ability to detoxify phenyl mercury (organic mercury). **Results:** The study showed that 8 mercury-resistant bacterial colonies could be isolated from the three soil samples. The bacteria were able to grow in LB broth containing 10 mg L^{-1} of phenyl mercury. Four isolates (AA, BB, CC and DD) were even able to grow in 40 mg L^{-1} of phenyl mercury. According to the identification tests, those bacteria were *Pseudomonas* sp. (AA, DD), *Pseudomonas aeruginosa* (BB) and *Proteus mirabilis* (CC). Testing of organic mercury against isolates of bacteria which are highly resistant to it in order to determine their detoxification capacity revealed that all four isolates could reduce levels of the compound in media, based on the results, starting from the highest was *Pseudomonas* sp. 74.99%, then *Pseudomonas aeruginosa* 60.23% and *Proteus mirabilis* 47.59% after 24 h of incubation. **Conclusion:** The study suggested that there are four bacteria that have potentials to remediate organic mercury contamination sites.

Key words: Organic mercury, remediation, phenyl mercury, mercury-resistant bacteria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural resources are one of the main profits of the state and direct income to the community. This results in the natural environment being greatly exploited, for instance, in order to get gold as a source of income. Besides the benefits which gold mining brings to the community, it also comes with disadvantages which are caused by uncontrolled activities. People exploit the environment to get the gold they want but sacrifice environmental health aspects, for example the use of mercury in the gold amalgamation process. The mercury waste from gold mining are processed and end up being released into the environment and this results in contamination of soil and water which is a source of livelihood for the community. In addition, the health of the surrounding community will be put at risk and there could be an emergence of a troubling disease.

Mercury is a very toxic element even though it is found in the body in small concentrations. But attention to public health is focused on the organic form of this compound which is its most toxic form. The biogeochemical cycle of mercury in the environment plays a role in regulating its toxicity, where the methylation process of direct, indirect mercury ions and degradation of methyl-mercury are the important keys to their toxicities, both in the mechanism of transformation by micro-organisms and the abiotic environment[1]. Organic mercury will be degraded to form mercury ions (Hg^{2+}) by the enzyme organomercury lyase and subsequently detoxified by mercury reductase to become volatile mercury (Hg^{o})[2].

Many reports revealed that habitats with high metal concentrations for years remain inhabited by microbial populations and activities even though they are in a lower density than that of uncontaminated habitats. This shows that these microbes can adapt to extreme conditions so it is assumed that bacteria can live in an environment contaminated with mercury with its specific defense mechanism[3,4]. Detoxification or bioremediation of bacteria against mercury is a form of defense mechanism against the compound in order to survive.

Mercury-resistant bacteria (MRB) strains have a set of genes called mercury-resistance (*mer*) operon, which is usually found in plasmids[5,6]. The mechanism for the bioremediation of mercury is through these genes and one of them is the *mer*B gene which encodes for the formation of the MerB enzyme. This protein will catalyse the breakdown of C-Hg bonds in organic mercury[7-9] to produce reduced carbon and mercury ions[5]. The process of breaking the C-Hg bond will release the Hg^{2+} ion which is usually followed by the reduction of the ion to Hg^{0} by the *Mer*A enzyme encoded by the merA gene[10]. These two stages make up a system of mechanisms for broad-spectrum MRB[11].

Fatimawali *et al.*[12] reported on the growth of various bacteria in traditional gold mining waste land in Bolaang Mongondow, North Sulawesi. The same research was conducted by Nofiani and Gusrizal[13], who stated that there was a growth of various bacteria in soil sediments which were formerly gold mining areas that had been contaminated with mercury in west Kalimantan. They identified 4 types of negative and positive-Grams bacteria with low, average and high resistance levels to mercury. In line with this opinion, Cursino *et al.*[14] stated that in mining areas in Brazil where mercury was used for gold extraction, there were bacteria which were resistant to mercury. Six proteobacterial isolates were also found in wastewater from factories using mercury in Germany and the Czech Republic[15].

Bacteria producing mercury-resistant genes have been widely exploited from the environment[16,17]. The use of mercury reductase and organomercury lyase to overcome the problem of inorganic and organic mercury pollution in the environment is in a very high demand. In a previous study, Fatimawali *et al.*[6] found bacteria which were resistant to inorganic mercury and survived in a medium with a concentration of $HgCl_2$ 40mg L^{-1}. Those bacteria were isolated from river estuaries of Manado Bay. The *mer*A gene was able to be isolated from *Klebsiella pneumoniae* isolate A1.1.1. The gene was cloned and able to overproduce recombinant *Mer*A protein. This protein can detoxify inorganic mercury. The protein obtained was successfully purified and characterized by having a 62 kDa molar mass[18]. Based on problems stated above, the purpose of this research was to identify organic MRB isolated from 3 sites near traditional gold processing locations in north Sulawesi and to test their abilities in detoxifying organic mercury.

MATERIALS AND METHODS

The experiment was conducted at Microbiology Laboratory, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, from March to June, 2018.

Sampling sites: Soil samples were obtained from 3 community mining process locations in Bolaang district that use mercury to extract gold from ore with more than 6 years of processing.

Isolation of bacteria resistant to organic mercury: Each of the soil samples were dissolved in sterile distilled water and then 100 µL was transferred into 10 mL sterile Nutrient Broth (NB) media. The media were incubated for 24 h at a temperature of 37°C. As much as 100 µL cultures were grown on Nutrient Agar (NA) media to get a single colony. Each single colony with different morphological appearance was grown on NB supplemented with 10, 20 and 40 ppm of phenyl mercury. Cultures grown on a medium with 10 ppm phenyl mercury were transferred onto NA containing 10 ppm phenyl mercury. Phenyl mercury level was determined using Cold Vapor Atomic Absorption Spectroscopy (CVAAS). Colonies that were able to grow on $\geq$10 ppm of phenyl mercury levels were considered as organic MRB, which were then selected and grown on slant NA agar supplemented with 10 ppm of phenyl mercury for further testing.

Identification of bacteria using 16S rRNA gene: The DNA of the bacteria were isolated using Genomic DNA Mini Kit (Geneaid). The isolated DNA was then stored at 2-8°C until used. Amplification of 16S rRNA gene was conducted using PCR combi block machine (Whatman Biometra Germany). Primer pairs bactF1 (forward) and uniB1 (reverse) were used to ampify the gene region. The amplification was performed by modifying the reagent composition and PCR condition. The PCR reagent composition included ddH_2O 20 µL, primer UniBl 30 pmol μL^{-1} 1,5 µL, primer BactFl 30 pmol μL^{-1} 1,5 µL, 2x MyTag HS Red Mix 25 µL, DNA template 2 µL. The following is the condition of PCR reaction: Initial denaturation 95°C for 180 sec, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 72°C for 30 sec and extention at 72°C for 90 sec. The final extention was performed at 72°C for 60 sec. Agarose gel 1% was used to separate the amplicons. Sharp bands indicated the success of amplification. The product of PCR together with the primer pairs were sent to 1Base Malaysia for sequencing.

Data analysis for bacterial identification: The sequencing results were firstly trimmed as much as 50 nucleotides at both ends to avoid the errorrness of the readings. The edited sequences were employed in Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to query for highly similar sequence. If the BLAST results showed highest identity percentage, the searched sequences were derived from expected species or genus[19].

RESULTS

Isolation of bacteria resistant to organic mercury: Table 1 showed the results of the isolation of organic MRB. From the tests, bacteria with isolate codes AA, BB, CC, DD, EE, FF, GG and HH were resistant to organic mercury. High MRB which survived at concentration of phenyl mercury 40 mg L^{-1} were isolates AA, BB, CC and DD. These isolates were subjected to molecular identification using 16S rRNA genes.

Detoxifying power of organic mercury-resistant isolates: Detoxifying power of the isolates that were highly resistant to phenyl mercury was shown in Fig. 1. Isolate AA showed the highest detoxifying power being able to reduce 74.99% of phenyl mercury. The second highest was isolate DD with 72.87%, followed by isolate BB with 60.23% and the lowest was isolate CC with 47.59%.

Identification of 16S rRNA genes: Figure 2 showed the PCR amplification result of 16S rRNA gene of the isolate AA, BB, CC and DD. All the isolates showed clear bands with the approximate size of 1500 bp.

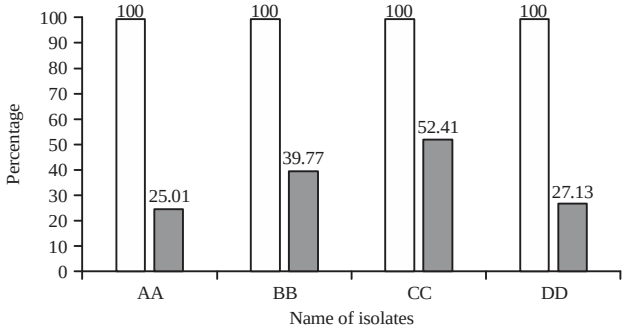

Fig. 1: Decreasing of phenyl mercury (%) in the media within 24 h

White color indicates the initial percentage of 40 mg L^{-1} phenyl mercury, gray color indicates the remaining percentage of 40 mg L^{-1} phenyl mercury

Table 1: Results of isolation of resistant bacteria to phenyl mercury

	Repetition 1				Repetition 2			
Bacterial code	5 ppm	10 ppm	20 ppm	40 ppm	5 ppm	10 ppm	20 ppm	40 ppm
AA	+	+	+	+	+	+	+	+
BB	+	+	+	+	+	+	+	+
CC	+	+	+	+	+	+	+	+
DD	+	+	+	+	+	+	+	+
EE	+	+	-	-	+	+	-	-
FF	+	+	-	-	+	+	-	-
GG	+	+	-	-	+	+	-	-
HH	+	+	-	-	+	+	-	-

Table 2: Results of BLAST analysis of 16S rRNA of the isolates resistant to phenyl mercury (40 mg mL^{-1})

Bacterial isolates	Species description	Max. Identity (%)	Query cover (%)
AA	*Pseudomonas* sp.	93	99
BB	*Pseudomonas aeruginosa*	99	99
CC	*Proteus mirabilis*	98	100
DD	*Pseudomonas* sp.	95	100

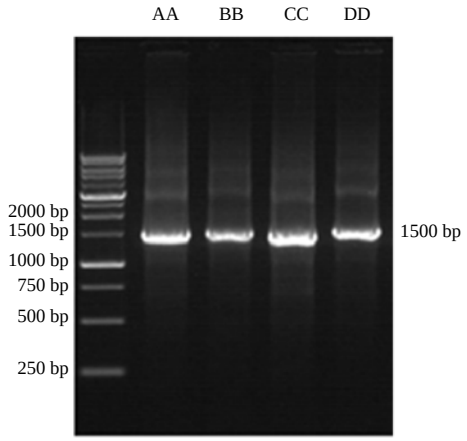

Fig. 2: Fragment of 16S rRNA of isolates AA, BB, CC and DD on 1% agarose gel

Identity search: Table 2 showed the identity search using BLAST on GenBank. The results showed that the bacterial isolates revealed very high identity with the searched sequences. Isolate AA had identity 93% with *Pseudomonas* sp., isolate BB 99% with *Pseudomonas aeruginosa*, isolate CC 98% with *Proteus mirabilis* and isolate DD 95% with *Pseudomonas* sp.

DISCUSSION

Tailings from gold mining operations can pose a threat to the environment. Traditional gold mining in Tanoyan Village, Bolaang Mongondow district, North Sulawesi province has been using mercury for gold extraction. The tailing wastes are contaminating the surroundings. However, some bacteria can withstand the presence of heavy metal in their surrounding. In order to survive, they develop and adopt various kind of detoxifying mechanism in deleterious environment. In laboratory setting, if the bacterial isolates can survive 24 h in medium containing 10 mg mL^{-1} of organic mercury, the isolates can be considered as tolerant. Enzyme that plays a role in detoxifying organic mercury (generally found as methyl-Hg, MeHg) is organomercury lyase (MerB)[18].

This research reported on the ability of eight organic mercury-resistant bacterial isolates obtained from the mercury contaminated soils in Tanoyan Village, Bolaang Mongondow district, North Sulawesi province to detoxify phenyl mercury (organic mercury). All isolates were able to reduce 10 ppm (10 mg mL^{-1}) of phenyl mercury in a period of 24 h. Only four isolates, designated as AA, BB, CC and DD were able to withstand up to 40 ppm of phenyl mercury (Table 1). According to Jaysankar[20], MRB are able to grow in 10 ppm mercury ($HgCl_2$). The highly resistant to mercury was capable to grow at 25 ppm mercury or higher[21]. However, no report has been obtained about the threshold concentration of organic mercury that can be tolerated by bacteria. Detoxification of organic mercury by MRB requires production of an enzyme, organomercurial lyase (MerB, encoded by *mer*B gene), that catalyzes the protonolysis of the carbon-mercury bond[22], to cleave Hg from organic residue. Kepel *et al.*[7] were able to isolate bacteria from sediment samples obtained from traditional gold mining at Tatelu village, North Minahasa district, North Sulawesi province. The bacterial isolates were able to grow in 10 ppm of phenyl mercury. They also were able to amplify *mer*B gene, which is responsible for encoding MerB protein.

In this study, mercury detoxification was carried out using MRB which have been identified as AA, BB, CC and DD isolates. Bacterial isolates were grown on nutrient broth (NB) media

containing a concentration of 40 ppm of phenyl mercury for a period of 24 h. CV-AAS method was used to carry out the analysis. The results of the analysis of mercury detoxifying power can be seen in Fig. 1. The 16S rRNA gene fragment of the four isolates were able to be amplified and produced sharp bands on agarose gel (Fig. 2). Present finding showed that based on 16S rRNA sequencing results, bacterial isolates which were able to withstand high concentration of phenyl mercury (40 ppm) were *Pseudomonas* sp. (isolate AA and DD), *Pseudomonas aeruginosa* (isolate BB) and *Proteus mirabilis* (isolate CC) (Table 1). The highest percentage reduction in phenyl mercury was carried out by *Pseudomonas* sp., as much as 74.9% (isolate AA) and 72.87% (isolate DD). *Pseudomonas aeruginosa* was able to reduce phenyl mercury by 60.23% and *P. mirabilis* by 47.59% (Fig. 1). Using 16S rRNA gene, Kepel *et al.*[7] were able to identify bacterial isolates *Bacillus cereus* and *Pseudomonas* sp., which were able to survive in 10 ppm of phenyl mercury. This implies that the genus *Pseudomonas* is the most tolerant to organic mercury. This is in accordance by the finding of Jan *et al.*[23] that several *Pseudomonas* species were found to exhibit the highest tolerance to both organic and inorganic mercury.

There are 3 response mechanisms when bacteria are exposed to mercury. The first one is by slowing cell metabolism down to delay cell growth or kill the cells. Secondly, it induces a mercury-resistant operon system to work in order to keep the cell alive even under stressful conditions and finally is the presence of a plasmid containing a mercury-resistant gene, which enters the cell. Bacterial isolates capable of living on media with mercury levels of 10-40 ppm are likely to make use of the second and even third response mechanisms to enable them to live on media with high mercury levels. Another factor that impacts on the decrease in mercury concentration in culture is the difference in the number of bacterial cells that grow in the media. This difference is caused by differences in optimum growth conditions such as pH and temperature of each type of bacteria[24].

CONCLUSION

Because not every microbe has the ability to reduce organic mercury compound, searching and exploiting them can be of benefit for bioremediation strategies in mercury contaminated environment. This current research showed that there were several microbes that were able to grow in very high concentration of organic mercury (40 ppm). These are *Pseudomonas* sp., *P. aeruginosa* and *Proteus mirabilis*. These bacteria can be investigated further for the presence of *mer*B gene encoding MerB protein which is responsible to cleave Hg from organic residue to produce a less toxic inorganic species Hg(II).

SIGNIFICANCE STATEMENT

This study discovers that *Pseudomonas* sp. is a very high organic mercury detoxifier species and it can be used for bioremediation of mercury-contaminated sites. However, because some Pseudomonades are pathogenic to humans, it is advisable to develop a safe method of mercury detoxification by using enzymatic methods which can be carried out in further studies.

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REFERENCES

1. Barkay, T. and I. Wagner-Dobler, 2005. Microbial transformations of mercury: Potentials, challenges and achievements in controlling mercury toxicity in the environment. Adv. Applied Microbiol., 57: 1-52.
2. Gupta, A., L. Phung, L. Chakravarty and S. Silver, 1999. Mercury resistance in *Bacillus cereus* RC607: Transcriptional organization and two new open reading frames. J. Bacteriol., 181: 7080-7086.
3. Konopka, A., T. Zakharova, M. Bischoff, L. Oliver, C. Nakatsu and R.F. Turco, 1999. Microbial biomass and activity in lead-contaminated soil. Applied Environ. Microbiol., 65: 2256-2259.
4. Summers, A.O., 2005. Metal Resistence Loci of Bacterial Plasmids. In: Frontier in Antimicrobial Resistence, White, D.G., M.N. Alekshun and P.F. McDermott (Eds.)., American Society for Microbiology, Washington DC., USA., pp: 165-173.
5. Benison, G.C., P. Di Lello, J.E. Shokes, N.J. Cosper, R.A. Scott, P. Legault and J.G. Omichinski, 2004. A stable mercury-containing complex of the organomercurial lyase MerB: Catalysis, product release and direct transfer to MerA. Biochemistry, 43: 8333-8345.
6. Fatimawali, F., B. Kepel and T.E. Tallei, 2015. Overproduction of mercuric reductase from mercury-resistant bacteria *Klebsiella pneumoniae* isolate A1.1.1. Indonesian J. Pharm., 26: 141-145.

7. Kepel, B.J., Fatimawali, I. Yusuf, R. Natsir and F. Badaruddin, 2012. Isolation and characterization of *mer*B gene in *Pseudomonas* sp. bacterium as organic mercury-resistantgene. J. Kedokteran Yarsi, 20: 69-80.
8. Miller, S.M., 1999. Bacterial detoxification of Hg (II) and organomercurials. Essays Biochem., 34: 17-30.
9. Omichinski, J.G., 2007. Toward methylmercury bioremediation. Science, 317: 205-206.
10. Fatimawali, 2016. Buku Ajar Toksikologi: Detoksifikasi Merkuri. Unsrat Press, Manado.
11. Barkay, T., S.M. Miller and A.O. Summers, 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev., 27: 355-384.
12. Fatimawali, B. Kepel, I. Yusuf, R. Natsir and F. Baharuddin, 2009. Bacterial population isolated from the soil of former mercury waste disposal in gold mining area in district of Bolaang Mongondow: A preliminary study. J. Kedokteran Yarsi, 17: 134-141.
13. Nofiani, R. and Gusrizal, 2004. Bakteri resistensi merkuri spectrum sempit dari daerah bekas Penambangan Emas Tanpa Izin (PETI) Mandor, Kalimantan Barat. J. Natur. Indonesia, 6: 67-74.
14. Cursino, L., S.M. Oberda, R.V. Cecilio, R.M. Moreira, E. Chartone-Souza and A.M.A. Nascimento, 1999. Mercury concentration in the sediment at different gold prospecting sites along the Carmo stream, Minas Gerais, Brazil and frequency of resistant bacteria in the respective aquatic communities. Hydrobiologia, 394: 5-12.
15. Von Canstein, H., S. Kelly, Y. Li and I.W. Dobler, 2002. Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions. Applied Environ. Microbiol., 68: 2829-2837.
16. Vetriani, C., Y.S. Chew, A.M. Miller, J. Yagi, J. Coombs, R.A. Lutz and T. Barkay, 2005. Mercury adaptation among bacteria from a deep sea hydrothermal vent. J. Bacteriol., 71: 220-226.
17. Poulain, A.J., S.M.N. Chadhain, P.A. Ariya, M. Amyot and E. Garcia *et al*., 2007. Potential for mercury reduction by microbes in the high arctic. Applied Environ. Microbiol., 73: 2230-2238.
18. Fatimawali, B. Kepel and T.E. Tallei, 2017. Overproduction of mercuric reductase protein expressed by synthetic merA gene and reduction of inorganic mercury $HgCl_2$. Biosci. Res., 14: 1253-1260.
19. Tallei, T.E. and B.J. Kolondam, 2015. DNA barcoding of Sangihe Nutmeg (*Myristica fragrans*) using *mat*K gene. HAYATI J. Biosci., 22: 41-47.
20. Jaysankar, D., 2004. Mercury-resistant marine bacteria and their role in bioremediation of certain toxicants. Ph.D. Thesis, Goa University, Goa, India.
21. De, J., N. Ramaiah and L. Vardanyan, 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Mar. Biotechnol., 10: 471-477.
22. Begley, T.P., A.E. Walts and C.T. Walsh, 1986. Mechanistic studies of a protonolytic organomercurial cleaving enzyme: Bacterial organomercurial lyase. Biochemistry, 25: 7192-7200.
23. Jan, A.T., M. Azam, I. Choi, A. Ali and Q.M. Haq, 2016. Analysis for the presence of determinants involved in the transport of mercury across bacterial membrane from polluted water bodies of India. Braz. J. Microbiol., 47: 55-62.
24. Smith, C.N., S.E. Kesler, B. Klaue and J.D. Blum, 2005. Mercury isotope fractionation in fossil hydrothermal systems. Geology, 33: 825-828.